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Research report

Morphine self-administration into the lateral septum depends on dopaminergic mechanisms: Evidence from pharmacology and Fos neuroimaging

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Abstract

Mice self-administer morphine into the lateral septum (LS), but the neuronal connections underlying this behaviour remain unknown. The present study tested whether the acquisition of intra-LS morphine self-administration depends on dopaminergic mechanisms. Mice were allowed to self-inject morphine (5 or 20 ng/50 nl) or vehicle directly into the LS using a spatial discrimination Y-maze task. Fos imaging was used to evaluate neuronal activation in cerebral structures directly connected to the LS or belonging to the dopaminergic system. The involvement of dopaminergic and opioidergic mechanisms was assessed by pre-treating naive mice peripherally with the D1 antagonist SCH23390, the D2/D3 antagonist sulpiride or the opiate antagonist naloxone before daily self-administration sessions. Mice acquired self-administration behaviour for intra-LS morphine that was associated with increased Fos expression in the ventral tegmental area (VTA), dorsal and ventral striatum and prefrontal cortex. Pre-treating animals with naloxone, SCH23390 or sulpiride completely prevented them from acquiring intra-LS morphine self-administration. All three antagonists consistently blocked Fos expression in the prefrontal cortex, but not in the VTA and striatum. Taken together, our results show that morphine self-administration into the LS depends on dopaminergic (D1 and D2/D3) and opioidergic mechanisms. Furthermore, they suggest that opioid peptides released in the LS could participate in regulating the activity of mesotegmental dopaminergic neurons.

Keywords: Intracranial self-administration; Septal area; Opiate receptors; Dopamine; D1, D2 and D3 receptors; Immediate early gene; *c-fos*; Nucleus accumbens; Mesocortical and mesolimbic pathways; Mouse

1. Introduction

The septal region was the first "reward centre" to be identified [50], but its implication in reward processes has been poorly explored further. Rats display intra-septal self-administration behaviour for both morphine [81] and met-enkephalin [82], suggesting that the septum participates in the reinforcing properties of opiates. This hypothesis is supported by anatomical data showing that mu, delta and kappa opioid receptors are expressed throughout the septal region [1,2,42]. Mice also self-administer morphine into the septal area, and this behaviour is acquired more rapidly with LS as opposed to MS injections [10]. Thus the lateral septum seems to play an important role within the septal area in supporting local morphine self-administration.

The lateral septum is connected to the ventral tegmental area (VTA) through both direct and indirect reciprocal projections [31,65], suggesting that the LS could support morphine self-administration by modulating mesolimbic activity [75]. The dopamine (DA) system arising from the VTA has received considerable attention as the major neurobiological substrate involved in mediating the rewarding properties of opiates [3,17,35,76,86], although not the only one [53,58]. Neurochemical data have consistently supported the existence of a functional opiate-DA interaction. Systemic injections of heroin [29] or of mu or delta opioid receptor agonists [29,59,60,80] increase the release of mesolimbic extracellular DA. Consistently, electrophysiological studies have identified a stimulant effect of opiates on dopaminergic neurons in the VTA [26,45].

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Electrical stimulation of the LS also regulates the firing rates of VTA neurons [41] suggesting that the rewarding properties of morphine, when injected into the LS, involve connections with the VTA and rely on dopaminergic mechanisms.

Our first experiment was designed to evaluate neuronal activation in brain regions that are sources or terminals of DA neurons in animals self-administering morphine into the LS, using *c-fos* labelling. A few additional regions were selected for being direct anatomical targets of the LS, in an attempt to assess the activity of LS projection neurons. Fos immunochemistry has been widely used as an indirect technique to map neuronal activation [18,30,70]. Furthermore, induction of Fos protein is known to be highly sensitive to morphine treatment under various experimental conditions [6,19,23,85].

Considering the proximity of the lateral septal region and the nucleus accumbens (NAc), specifically the shell part of the NAc (AcbSh), which is also known to support opiate self-administration [13,14,25,51], the reinforcing properties of intra-LS morphine may result from diffusion towards this structure. In the present study, the anatomical specificity of intra-LS morphine self-administration was assessed by comparing the performance and the pattern of brain Fos expression of animals receiving 20 ng morphine into the LS with these of animals receiving the same dose of morphine into the dorsal part of the AcbSh.

If morphine self-administration into the LS involves dopaminergic mechanisms, pre-treating animals with DA receptor antagonists should prevent its acquisition. Among DA receptors, D1 and D2 subtypes might not contribute equally to the rewarding and conditioning properties of opiates [40,43,77]. Moreover, in case of local injections, the balance between D1 and D2 mechanisms may differ depending where morphine is infused [14]. We designed a second series of experiments to test the effects of pre-treating mice with either the selective D1 antagonist SCH 233390 or the D2/D3 antagonist sulpiride on the acquisition of intra-LS morphine self-administration, and we compared them with the effects of pre-treatment with an opiate receptors antagonist (naloxone), expected to prevent morphine self-administration. Immunochemistry for Fos protein is particularly sensitive to dopaminergic manipulations [66,69]. We used this technique in the present study to evaluate the consequences of previous pharmacological treatments on neuronal activity in DA-related cerebral structures and anatomical targets of the LS.

2. Methods

2.1. Animals and surgery

Ninety-one male mice of the inbred BALB/cByJICO strain obtained from Iffa-Credo (Lyon, France) were used. At 10 weeks of age, they were housed individually with ad libitum access to food and water in a temperature-controlled room (23 ± 1 °C) maintained on a 12 h light/dark cycle (lights on at 7.00 a.m.). Mice were aged 14–18 weeks and weighed about 28–32 g at the beginning of the experiments.

Under deep anaesthesia (Avertin, 300 mg/kg ip; local Xylocaine, 5%), animals were implanted unilaterally, in a counter balanced left and right order, with one guide cannula (8 mm long, o.d. 0.460 mm, i.d. 0.255 mm) into the dorsal LS or into the dorsal AcbSh. The stereotaxic coordinates were for the LS: antero-posterior (AP) + 0.80 mm; lateral (L) ± 0.4 mm; vertical (V) −1.60 mm; for the AcbSh: AP + 1.40 mm; L ± 0.6 mm; V −2.8 mm. To minimize tissue damage, the tip of the guide cannula was positioned 1.5 mm above the targeted structure. The cannula was anchored to the skull with two stainless steel screws and rapid-setting acrylic dental cement. Patency was maintained by inserting a stylet that fitted the length of each guide cannula. Mice were allowed 10 days to recover from the operation. All surgical and experimental procedures were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Materials and experimental procedure

2.2.1. Intraseptal self-injection procedure

On each day of the experimental period, a stainless-steel injection cannula (outer diameter 0.229 mm, inner diameter 0.127 mm) was inserted into the targeted structure through the guide cannula and held in a fixed position by means of a small connector. The injection cannula was connected by flexible polyethylene tubing to the micro-injection system, which housed a 5 μl Hamilton syringe. The tip of the injection cannula projected 1.5 mm beyond the guide-cannula. By interrupting one photocell beam in the Y-maze (see Section 2.2.2) mice could obtain an infusion of morphine sulphate dissolved in Ringer solution. Intracerebral injections were carried out using an automatic computer-controlled apparatus which provided, via a micro-vernier system, a precise and highly reproducible descent of the microsyringe piston. Each self-infusion (50 nl) lasted 4 s. Normal drug flow was verified visually both before and after each session for each animal. Any movement of the animals in the Y-maze was detected by an optical system. This information was transmitted to a micro-computer which rotated in turn the injector in the same direction as the animal's movement. This process avoided the twisting of the flexible tubing; consequently, self-administration was studied in freely moving mice.

2.2.2. Behavioural protocol

Self-administration behaviour was studied in a grey Plexiglas Y-maze, the two arms of which were separated by an angle of 90°. The stem and the arms were 31 cm long and 12 cm high. The starting box (14 cm × 8 cm) was separated from the stem by a sliding door. Sliding doors were also located at the entrance of each arm. A photo-electric cell was situated 6 cm from the end of each arm. By interrupting the photocell beam in one of the two target arms, mice could trigger an intracranial injection (reinforced arm); detection of movement in the other arm had no effect (neutral arm).

During a first habituation session, no injection was delivered. During the following sessions, a trial started when a mouse was placed in the start box. After 1 min, the door to the stem was opened. In each group, half of the animals were assigned to go to the right arm to trigger intracerebral injections whereas the others were assigned to the left arm. Each daily session was composed of 10 trials separated by a 1 min intertrial interval. Therefore a maximum of 10 injections could be obtained per subject per daily session. During the first four trials of the first session only, the animal having made an error in choosing the neutral arm was allowed to enter the other arm and obtain an injection of morphine. From the fifth trial onward, if the mouse made an error, the chosen arm was closed. After a 10 s confinement, the mouse was removed and replaced directly into the start box for the following trial. The number of self-administrations per daily session was noted and automatic equipment, triggered by opening the door to the stem, recorded the latency to enter the reinforced arm (injection latency) or the neutral arm for each subject.

The present study consisted of two main experiments.

Experiment I: acquisition of morphine self-administration into the LS or AcbSh. Acquisition of intra-LS self-administration for two doses of morphine [5 ng (6.5 pmol) or 20 ng (26 pmol) per injection of 50 nl] was studied in two independent groups of mice, while a control group was exposed to intra-LS vehicle (Ringer solution) during 6 days ($n = 8$ implanted animals per group). Morphine sulphate (donated by Francopia, Sanofi Synthelabo laboratories) was dissolved in sterile Ringer solution (Meram, France) and administered intracerebrally into the LS (pH 6.1–6.2).

Acquisition of self-administration behaviour for intra-AcbSh morphine at 20 ng/50 nl or vehicle (Ringer) was studied as an anatomical control in two independent groups of mice during 6 days ($n = 8$ per group).